

Acute Renal Failure Caused by Nephrotoxins

by Donald E. Oken*

Renal micropuncture studies have greatly changed our views on the pathophysiology of acute renal failure caused by nephrotoxins. Formerly, this type of renal insufficiency was attributed to a direct effect of the nephrotoxins on tubule epithelial permeability. According to that theory, glomerular filtration was not greatly diminished, the filtrate formed being absorbed almost quantitatively and nonselectively across damaged tubule epithelium. Studies in a wide variety of rat models have now shown glomerular filtration to be reduced to a level which will inevitably cause renal failure in and of itself. Passive backflow of filtrate across tubular epithelium is either of minor degree or nonexistent even in models where frank tubular necrosis has occurred. This failure of filtration cannot be attributed to tubular obstruction since proximal tubule pressure is distinctly subnormal in most models studied. Instead, filtration failure appears best attributed to intrarenal hemodynamic alterations. While certain facts tend to incriminate the renin-angiotensin system as the cause of the hemodynamic aberrations, others argue to the contrary. The issue is under active investigation.

Although the clinical syndrome of post-traumatic acute renal failure was well recognized early in World War II, its pathogenesis remained the subject of debate for many years thereafter. Since the glomeruli and renal vessels of subjects dying with this syndrome appear entirely normal to histologic examination and since renal failure persists long after the patients' blood pressure and cardiac output have returned to normal, glomerular filtration was widely assumed to be essentially unimpaired. Tubular injury with concomitant interstitial edema and intraluminal pigment or debris, the major histopathologic findings, were felt by most early investigators to be the key etiologic factors. Accordingly, it was suggested that a normal volume of glomerular filtrate was formed but then absorbed, passively and totally unselectively, across necrotic epithelium which no longer served as an effective passive absorption barrier. Tubular debris was envisaged as a cause of tubular obstruction and high intratubular pressure, the latter purportedly augmenting the rate of nonselective tubular absorption and/or reducing or nullifying effective filtration pressure. These same mechanisms were believed to be operative in acute renal failure

induced by poisoning, blackwater fever, heat-stroke, sepsis and hemolytic reactions.

It was soon pointed out, however, that tubular necrosis and luminal debris are frequently either absent or so minor as to be unlikely contributors to the pathogenesis of renal failure (1-3). Furthermore, renal blood flow was repeatedly shown to be reduced to a degree that, if due to preglomerular arteriolar constriction, would inevitably lower glomerular capillary pressure below the level needed to sustain filtration, (4-6). Nevertheless, the obstructive and passive backflow theories of pathogenesis persisted and were so widely accepted that the syndrome was given the name, acute tubular necrosis. Until renal micropuncture was used in our laboratory to study this problem some twelve years ago, however, the exact role played by each possible functional aberration was purely conjectural.

Renal micropuncture affords an excellent means of evaluating the various theories of the pathogenesis of acute renal failure. The obstructive theory can be tested by measuring intratubular pressures manometrically. One can derive the glomerular filtration rate (GFR) and fractional water absorption of individual nephrons from the tubule fluid:plasma inulin concentration ratio but this requires absolute confidence that significant amounts of this normally unabsorb-

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able indicator have not leaked across a necrotic epithelial cell wall. It is possible, however, to obtain much the same information (albeit only qualitatively) by using observations which are direct and unequivocal. On examining the kidney surface under a dissecting microscope, for example, it is evident whether the superficial nephrons are full of fluid or are collapsed. Tubule fluid absorption rates can be estimated directly by observing the rate at which injected isotonic saline droplets are absorbed from the proximal tubule. Injecting a small drop of stained mineral oil, one can clearly discern whether the fluid in a given tubule flows briskly, slowly, or not at all. Since any fluid which has not been absorbed up to a given site in a tubule will be propelled onward by new filtrate reaching that point, the absence or near absence of flow in early proximal tubule segments of fluid filled nephrons is clear and sufficient evidence that filtration is massively reduced. GFR and water absorption can, therefore, be measured qualitatively without making any assumptions as to the tubule's inulin permeability. Some of our experiments utilizing renal micropuncture techniques in a variety of models of experimental acute renal failure in the rat will be described and our present thoughts on pathogenesis summarized.

The first renal micropuncture study of the pathophysiology of acute renal failure utilized 12 mg/kg body weight (BW) HgCl_2 as the etiologic agent (7). Single-nephron GFR, tubule fluid flow rates, proximal tubule sodium and water absorption, and intratubular pressures were measured at different times up to 48 hr after mercury injection. Within 6 hr, glomerular filtration had fallen by one half. Cell debris was seen to flow down some tubules with no apparent tendency to aggregate, but intratubular pressure remained normal throughout this period (Fig. 1). Therefore, single-nephron GFR and tubule fluid flow decreased progressively until, in the succeeding hours, they became too slow to be quantified (Fig. 2). With grossly depressed flow in the proximal tubule, debris sometimes coalesced into potentially occlusive intraluminal masses. Nevertheless, proximal tubule hydrostatic pressure actually fell (Fig. 1) and the debris could frequently be made to flow out of the tubule when fluid was injected intraluminally at normal tubule hydrostatic pressure. The fall in GFR could not, therefore, be attributed to the presence of such material. Rather, it appeared that any element of obstruction which developed was actually the result

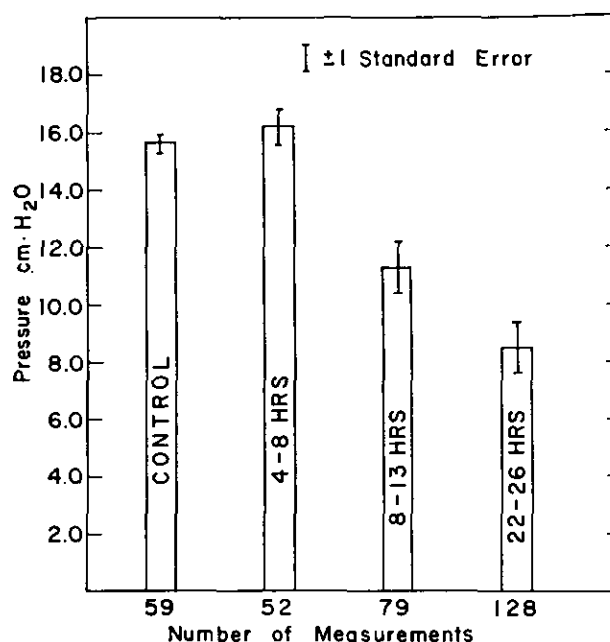


FIGURE 1. Hydrostatic pressure in proximal tubules measured after the injection of HgCl_2 , SC, 12 mg/kg BW. The times given are hours after injection. From Flanigan and Oken (7) with permission of the Journal of Clinical Investigation.

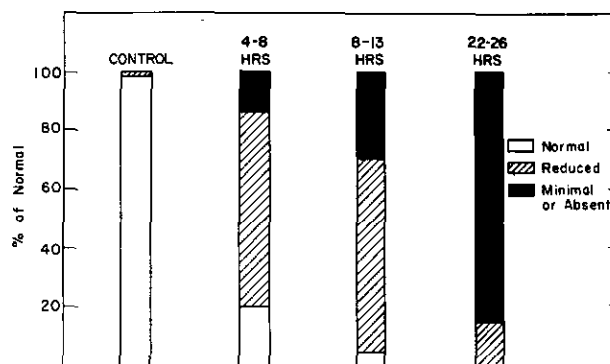


FIGURE 2. Qualitative estimates of proximal tubule flow rate in rats given HgCl_2 , SC, 12 mg/kg BW. From Flanigan and Oken (7) with permission of the Journal of Clinical Investigation.

of filtration failure, greatly decreased flow permitting aggregation of material which seemingly would have been propelled out of the tubule if the GFR had been well maintained. While GFR was moderately brisk, fractional water absorption in the proximal tubule was essentially normal. Water absorption could no longer be quantitated, as filtration fell to vanishingly low levels but,

judging from the extremely slow rate at which segments of injected saline were absorbed, must have been grossly reduced. This study indicated, therefore, that impaired glomerular filtration was the prime abnormality leading to this variety of acute renal failure, and that tubular obstruction (when it occurred) was a secondary phenomenon. Increased tubular permeability to salt and water could not be demonstrated.

Very similar results were obtained in a model of acute renal failure induced with intramuscular glycerol injections (8). This model, characterized by rhabdomyolysis, acute plasma volume depletion, hemolysis, and myohemoglobinuria has many similarities to the human crush syndrome, and is probably the most meaningful of the experimental models available today (9).

Soon after the injection of glycerol the majority of nephrons on the kidney surface became totally collapsed, an indication of filtration failure (8). Stained oil droplets injected into fluid filled nephrons usually remained at the injection site for prolonged periods of time, again showing flow (and thus filtration) to be exceedingly slow or totally absent (Fig.3). The intratubular pressure of fluid filled nephrons was grossly subnormal

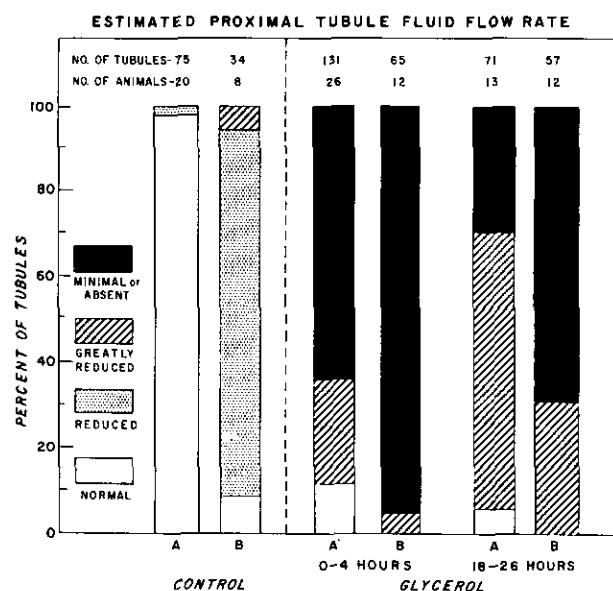


FIGURE 3. Qualitative estimates of proximal tubule flow rate in rats given 50% glycerol, 10 ml/kg BW. Columns A and B refer to normally hydrated rats and water-deprived rats, respectively. The times shown are hours after injection. From Oken et al. (8) with permission of the Journal of Clinical Investigation.

(Fig. 4), an indication that obstruction was not responsible for the observed impairment of filtration. External compression such as might occur with interstitial edema could not have been responsible for their collapse, since it was possible to inject fluid into collapsed tubules at pressures below 5 cm H₂O. Studies performed on the day after glycerol injection showed a smaller proportion of nephrons totally devoid of flow, but single-nephron GFR and flow rates in the very best functioning nephrons still were very low. Proximal tubule hydrostatic pressure and fractional water absorption, where quantifiable, remained distinctly subnormal. Droplets of isotonic saline injected into the lumens of collapsed tubules were absorbed at a rate so slow as to be barely appreciable to the eye. The fact that nephrons with grossly reduced glomerular filtration remained fluid-filled also served as direct evidence of maximally depressed fluid absorption. Thus, as with the mercury model, this study showed glomerular filtration failure in tubules whose pressure was low and fluid absorptive capacity was markedly reduced. Evidence to support the passive back-flow hypothesis of acute renal failure could not be evinced.

It is intriguing that acute renal failure persists for such a long time after all the antecedent hemodynamic abnormalities have returned to normal. It has thus been suggested that recovery

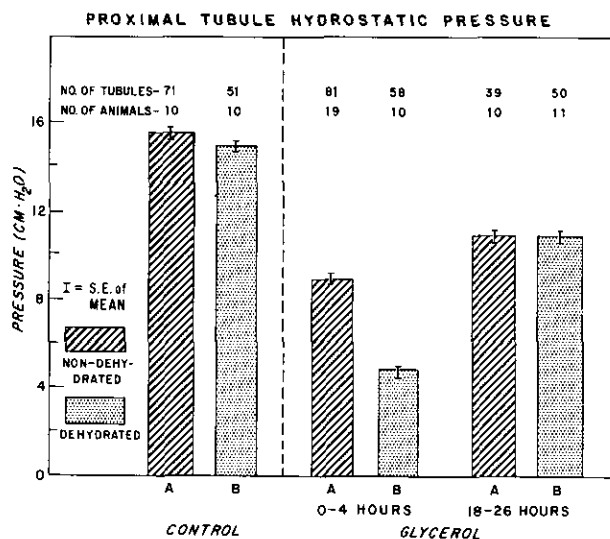


FIGURE 4. Proximal tubule hydrostatic pressures in glycerol-injected rats. See Figure 3. From Oken et al. (8) with permission of the Journal of Clinical Investigation.

reflects the repair of necrotic tubular epithelium, the release of obstruction (which may not have been apparent so long as filtration was exceedingly low), or the resolution of interstitial edema which characteristically develops during the oliguric period. In an attempt to characterize the events associated with recovery, we again turned to glycerol-induced acute renal failure (10). Glycerol was administered at the same dose and route as in the initial study (8), and experiments were preformed when the animals' BUN concentration had fallen at least 20% below the peak value. Some animals were studied when their GFR was essentially normal while others were still severely azotemic. Regardless of the degree of recovery achieved by a given rat, two populations of nephrons were found: one with a perfectly normal GFR and the other with a filtration rate which was far too low to permit quantitation. Nephrons with intermediate GFR values were rarely found. As shown in Figure 5, an excellent inverse correlation held between the proportion of filtering nephrons on the kidney surface and the whole kidney inulin clearance rate. Thus, recovery reflected the progressive recruitment of increasing numbers of previously nonfiltering nephrons rather than a gradual increase in function of the entire nephron complement. The proximal tubule hydrostatic pressure of functioning nephrons was normal while that of collapsed nephrons was decidedly low; no group of nephrons had a high intratubular pressure to suggest that obstruction might delay the recovery of filtration.

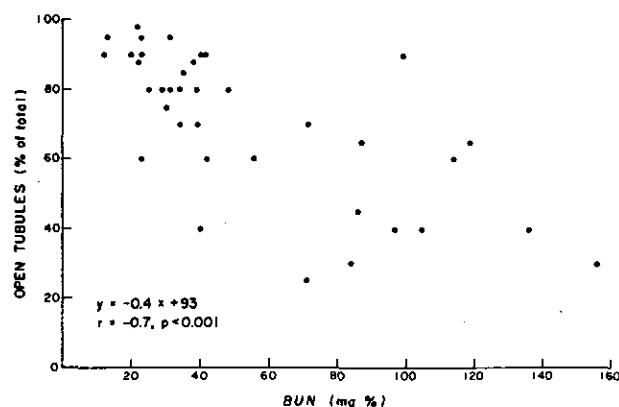


FIGURE 5. The percentage of functioning nephrons in kidneys of rats recovering from glycerol-induced renal failure shown as a function of degree of recovery (BUN). An excellent correlation is seen, $p < 0.001$. From Oken et al. (10) with permission of the Journal of Clinical Investigation.

Whether filtering or not, all nephrons displayed significantly reduced—not augmented—water absorption. It thus seemed unlikely that recovery relates directly to the repair of tubule injury, a phenomenon which would be expected to give a wide spectrum of filtration and flow rates as tubular integrity improved. As manifest by the homogeneity of the values obtained in each of the two nephron populations, such a spectrum of nephron function did not exist. Interstitial edema, although severe, did not correlate with the degree of recovery attained, a kidney weight three times normal being compatible with complete return of renal function to normal levels (Fig. 6). The rapid normalization of the GFR of some nephrons while those adjacent were filtering little if any fluid suggested that failed filtration reflected a local, rather than a circulating, pathogenetic mechanism. More will be said on that thought later.

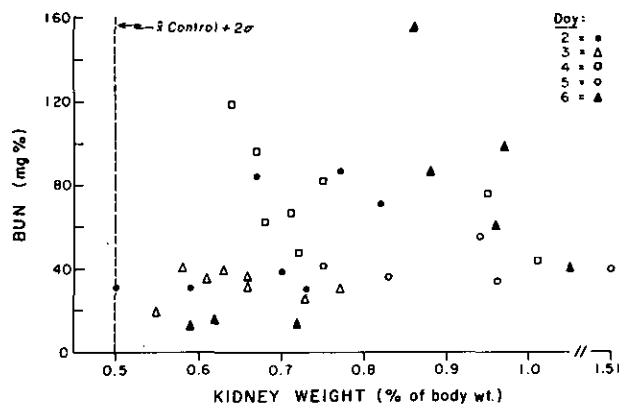


FIGURE 6. The (lack of) correlation between kidney weight, an index of interstitial edema, and return of renal function in rats recovering from glycerol-induced renal failure. From Oken et al. (10) with permission of the Journal of Clinical Investigation.

The conclusions drawn in the micropuncture studies just described were supported by comparable experiments reported by Barenberg (11), Ruiz-Guinazu (12), Jaenike (13), Cirkseña (14), and Henry (15) and their colleagues on a variety of experimental models. Steinhausen (16) and Bank (17) and co-workers did, however, find evidence for tubular leakage in low dose (approximately 4 mg/kg) mercury poisoning, but our studies (18) suggested that such findings may have been artifactual. Dramatically illustrating that conclusion, rats given 1% saline in place of

tap water before the same mercuric chloride challenge failed to develop significant renal insufficiency. Their inulin clearance values were close to normal 24 hr after the injection of mercury (18). Nevertheless, their kidney showed tubular epithelial necrosis which was just as severe as, although slightly less extensive than, that displayed by their water drinking counterparts (19). The wide zone of frank tubular necrosis in the kidney of the animals drinking saline solution (Fig. 7) should have provided ample opportunity for passive backflow of glomerular filtrate to occur. Evidently, in view of the essentially normal blood urea nitrogen levels and kidney inulin clearances obtained, neither inulin nor urea was absorbed abnormally. If the passive backflow theory is not applicable to kidneys with frank necrosis of the entire terminal portion of the proximal tubule, it is highly unlikely to be a major determinant of other types of acute renal failure in which tubule damage is far less severe.

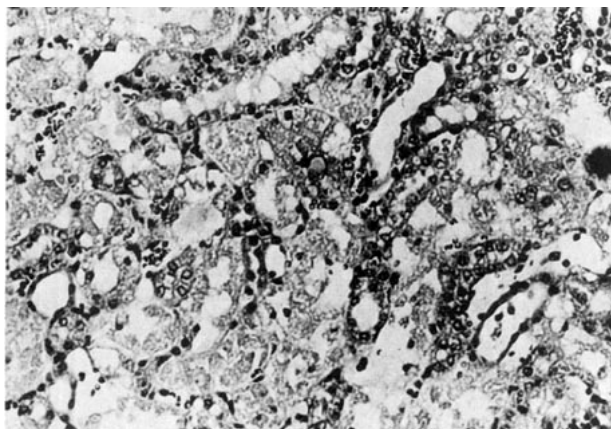


FIGURE 7. High power photomicrograph illustrating severe tubule necrosis in a rat 48 hr after injection with HgCl_2 , 4.7 mg/kg BW. Despite severe tubular necrosis, this rat's inulin clearance was 87% of normal original magnification, 200 \times .

To reiterate, most varieties of experimental acute renal failure studied in our own and other laboratories reflect a primary impairment of glomerular filtration which cannot be attributed to tubular obstruction; nonselective passive reabsorption of filtrate appears not to be an essential concomitant. Filtration failure in the absence of increased intratubular pressure must then reflect either a low glomerular filtration pressure or grossly reduced glomerular capillary permeability. We set out, therefore, to try to delineate the

mechanisms which might be responsible for either abnormality. But where should we start?

Dehydration is a well known factor predisposing to the development of both experimental and human acute renal failure (9). This effect has been attributed to a vasomotor action of antidiuretic hormone (ADH) *per se*, and/or increased urine concentration with slow flow through the distal segment of the nephron promoting tubule obstruction (20). We, therefore, decided to see whether ADH and urine flow rate did indeed influence the development of acute renal failure. As shown earlier (8), dehydration greatly increases the severity of glycerol-induced myohemoglobinuric renal failure in the rat. If, therefore, this effect were mediated through the concentrating mechanism, animals with complete hypothalamic diabetes insipidus should be effectively protected from renal insufficiency. Such animals, totally lacking ADH, excrete volumes of maximally dilute urine equivalent to 80% of their body weight each day, yet they were not protected. Rather, they developed a degree of renal insufficiency intermediate between that expected in normally hydrated and deliberately dehydrated rats subjected to the same challenge (21). On micropuncture, the renal functional abnormalities obtained were identical to those seen in normal rats given the same dose of glycerol. Accordingly, the development of this model of acute renal failure is not dependent on the renal concentrating mechanism, and factors other than the antidiuretic hormone titer, urine osmolality, and urine flow rate must be responsible for the effect of dehydration on the response to a renal failure challenge.

Having failed to find an essential role of the concentration mechanism in the pathogenesis of acute renal failure, it was almost inevitable that the renin-angiotensin system would be studied next. First, we were looking for a potent renal vasoconstrictor mechanism. Second, Goormaghtigh had suggested many years earlier (22) that the juxtaglomerular apparatus might serve as a regulator of glomerular filtration both under physiologic conditions and in acute renal failure. Third, plasma renin titers have been repeatedly demonstrated to be elevated in human and experimental acute renal failure (23,24). Since Gross and co-workers (25) had reported that plasma renin levels of rats can be suppressed by long term saline loading, we employed this maneuver to see whether rats given 1% saline solution in place of drinking water over a period of

one month before being put at risk of acute renal failure would be protected. Challenged with either glycerol or mercury, animals so treated were indeed almost completely protected from renal insufficiency (26,27). Comparable protection was documented by other investigators using dichromate (15) or uranyl nitrate (28) as the nephrotoxic agent. Micropuncture studies showed the rapid reversal of an initial period of filtration failure and tubular collapse after glycerol administration (9). As had been reported by Gross (25), plasma renin titers were maximally suppressed (29), and it was tempting to suggest that the protection observed related to renin depletion. If so, the renin system may be assumed as the primary cause of renal dysfunction in this syndrome. Long-term saline loading has widespread effects other than renin depletion (30,31); however, and it seemed necessary to test the protective role of renin depletion in other experiments before making such an assumption.

Accordingly, a study was undertaken to determine whether it would be possible to prevent rats from developing mercuric chloride or glycerol-induced acute renal failure by specific immunization against renin (29). Although biologically effective immunization was achieved, no significant prophylactic effect could be demonstrated. What is more, both active immunization (unpublished data) and passive immunization with potent anti-angiotensin antibody produced in rabbits (32) offered no better protection of renal function. Thus, although the "saline" experiments had strongly suggested that the renin-angiotensin system plays a key role in the pathogenesis of acute renal failure, the immunization studies were unable to support that possibility. In view of findings summarized elsewhere (32), it seems likely that any role played by the angiotensin system is most apt to be directly at the afferent arteriole, and large molecular weight immunoglobulins might not be able to reach the effector site within the muscle wall. Thus, our experiments, while not supporting a role of angiotensin, by no means controverted that possibility and the renin-angiotensin system remained a potentially important suspect as a pathogenetic factor in acute renal failure. Further experimentation was needed.

Lever and his colleagues (33) obtained no more success in preventing glycerol-induced acute renal failure with chemical inhibitors of the angiotensin system than we had obtained with immunization. Here too, however, even the low

molecular weight inhibitors may not have been able to reach the effector site to neutralize an angiotensin effect and, even if they did, might be present in minor concentration relative to the exceedingly large amount of renin present in the juxtaglomerular apparatus and arteriolar wall. We, therefore, decided on a different approach, searching for a means of preventing acute renal failure by a means other than long-term saline loading. This approach was taken in the belief that if two totally distinct means of preventing acute renal failure were both characterized by renal depletion, a role of the renin-angiotensin system would be more substantively suspected.

Hayes and co-workers provided the lead we needed, having shown that rats recovering from myhemoglobinuric acute renal failure do not develop significant renal dysfunction when rechallenged with a second intramuscular glycerol injection (34). If, however, the vasomotor consequences in this particular model depend (if only in part) on the initial release of some as yet undetected substance from injured muscle, depletion of such material by the first injection might explain these animals' refractoriness in and of itself. To simplify the problems of interpretation and be sure that this phenomenon is not peculiar to the glycerol model, a protocol was designed in which rats were given either an intramuscular injection of glycerol or mercuric chloride subcutaneously. When the animals' BUN concentration had fallen by some 50% or more below the peak value, the group given mercuric chloride as the first injection was rechallenged with glycerol; the animals first injected with glycerol received mercuric chloride. Both groups of rats proved to be highly refractory to the second renal failure challenge (Fig. 8). Unlike chronically salt-loaded rats, however, such animals had a normal or elevated renal renin titer at the time of their second injection (35). Evidently, renal renin depletion is not essential to the protection from renal dysfunction that these rats so clearly displayed. Nor did protection relate to an insensitivity of their vasculature to angiotensin (36) or an inability to release renin normally in response to hemorrhage or isoproterenol infusions (J.S. Carvalho and D.E. Oken, unpublished observations). Thus, the concept that the renin-angiotensin system serves as the key mediator of acute renal failure is far from proven and, indeed, may be seriously questioned.

Theoretically, the near cessation of glomerular filtration in acute renal failure might be the result of decreased glomerular capillary per-

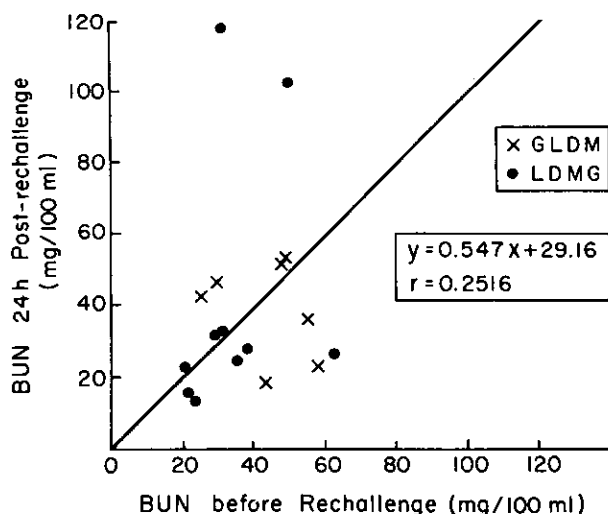


FIGURE 8. BUN concentration 24 hr after a second acute renal failure challenge in rats recovering from prior renal failure. GLDM refers to rats with glycerol induced renal failure given mercury as the second challenge; LDMG represents the order of injection. Eight rats showed a small decrease in BUN concentration, and 7 a small rise. Only two of the 17 rats studied had a major change in renal function. From Oken et al. (35) with permission of Nephron.

meability rather than low glomerular capillary pressure. Such a mechanism has received relatively little attention until recently (37,38), and further studies of glomerular capillary permeability are needed.

The potential role of prostaglandins in the pathogenesis of acute renal failure has only recently been appreciated. Literature on the effect of prostaglandins on normal renal function has expanded rapidly in recent years and is beautifully summarized by Zins (39). These agents have potent effects on both total renal blood flow and its intrarenal distribution. Barger, for example, has shown that PGA_2 in fused into the renal artery of conscious dogs results in the redistribution of blood flow from the outer medulla to the renal cortex (40). PGA in renal tissue and blood, measured by radioimmunoassay, increases after acute renal artery clamping (41). PGA_1 in subhypotensive amounts has been reported to be capable of reversing the oliguria and depressed renal plasma flow produced by angiotensin II (42), and both the E_2 and A_2 prostaglandin moieties are capable of reversibly inhibiting the vasoconstrictor effects of renal nerve stimulation and injections of angiotensin or norepinephrine. Even the protective effect of longterm salt loading might be partly exerted through the

prostaglandin system since this maneuver also augments prostaglandin synthesis (31). In view of the well established renal vasodilator effect of prostaglandins and their ability to reverse induced vasoconstriction, it is possible that the vasomotor aberrations seen in acute renal failure might reflect, at least in part, a deficiency in renal prostaglandin synthesis, metabolism, release, or delivery. Indeed, Fine (43) has suggested on purely theoretic grounds that renal prostaglandin depletion might be responsible for the genesis of acute renal failure. In this regard, Gerhard and Mulrow (44) reported low renal concentrations of prostaglandin-A in rats subjected to glycerol-induced renal failure 24 hr earlier. The reduction in prostaglandin concentration correlated well with the degree of renal insufficiency induced. Since, however, indomethacin lowered prostaglandin titers even further without affecting the severity of renal failure, the authors suggested that the reduced prostaglandin content was secondary to renal damage and did not itself influence the severity of renal failure. Torres and co-workers (45), by contrast, found increased concentrations of prostaglandin in kidneys of rats and rabbits with glycerol or mercuric chloride induced acute renal failure. The same laboratory has reported that prostaglandin depletion resultant on indomethacin treatment aggravates the development of acute renal failure in the rat (46), however. The suggestion that prostaglandins may be involved in the genesis of acute renal failure by some as yet undefined mechanism thus finds some support, although indomethacin might tend to worsen renal failure by other means than prostaglandin depletion.

Present evidence indicates that renal prostaglandins are synthesized in the medulla but have potent effects on the cortical circulation. Only small amounts appear to be produced in the cortex (47) which is very rich in 15-hydroxy-dehydrogenase, the major metabolizing enzyme for prostaglandins (48). There are no vascular or lymphatic channels directly connecting the medulla with the mid- and outer cortex of the kidney, and it seems unlikely that the prostaglandins reach the cortex by diffusion because of the rapidity with which they act and the likelihood that they would be degraded by cortical dehydrogenases en route. Henle's loop does lead directly from the medulla to the cortical segment, however, and it is at least reasonable to suggest that this may be the pathway through which prostaglandins synthesized in the renal medulla travel without being enzymatically degraded (49). Re-

cent studies by Kauker (50) and Frolich and co-workers (51) indicate that the prostaglandins are, indeed, added to the pre-urine intrarenally, probably through the loop of Henle. Entering Henle's loop in the normal kidney, prostaglandins would then be present in distal tubule fluid bathing the macula densa—the presumed site of glomerular arteriolar regulation—and might thus serve a modulating function on filtration and blood flow. Such a feedback system could operate in the normal kidney regardless of the proportion of long loops of Henle, since even the outer medulla contains significant amounts of prostaglandin (48). If, however, filtration stops completely because of overwhelming cortical vasoconstrictor stimuli in the prodromal phase of acute renal failure, flow along the loop also stops and prostaglandins would no longer reach the macula densa (49). The prostaglandin feedback loop would then be opened, constrictor mechanisms remain unchecked and filtration failure might thus be perpetuated. At present there is no firm experimental evidence to support such a concept. In a previous study of low-dose mercury poisoning, however, we have found that nephrons which were filtering virtually no fluid and had minimally increased intratubular pressure began to filter at a rate approximately 80% of normal once flow had been induced by injecting isotonic saline solution into their proximal tubules at physiologic hydrostatic pressure (18). A feedback system between tubule fluid flow and filtration was postulated but was not pursued further, and other models of acute renal failure have not yet been examined in this light. Whether restoration of flow in such tubules caused the return of glomerular filtration as result of the resumed delivery of prostaglandins to the macula densa or of other factors remains to be determined. The finding is, however, in keeping with the hypothesis of a complex tubular/glomerular filtration feedback mechanism which may be associated with neural factors, the renin-angiotensin system, and availability of prostaglandins reaching the glomerular vascular pole.

We are now in a situation where the events attendant on experimental acute renal failure are reasonably well known. There still remains the task of identifying the mechanisms mediating these events. We shall follow the clues we have described today to achieve that end, but it is evident that new study techniques will need to be applied.

REFERENCES

1. Finckh, E. S., Jeremy, D., and Whyte, H. M. Structural renal damage and its relation to clinical features in acute oliguric renal failure. *Quart. J. Med.* 31: 429 (1962).
2. Sevvitt, S. Pathogenesis of traumatic uremia—a revised concept. *Lancet* (2): 135 (1959).
3. Oken, D. E.: On the passive back flow theory of acute renal failure. *Am. J. Med.* 58: 77 (1975).
4. Hollenberg, N. K., et al. Acute oliguric renal failure in man. Evidence for preferential renal cortical ischemia. *Medicine* 47: 455 (1968).
5. Conn, J. L., Jr., Wilds, L., and Helwig, J. A study of the renal circulation tubular function and morphology, and urinary volume and composition in dogs following mercury poisoning and transfusion of human blood. *J. Clin. Invest.* 33: 732 (1954).
6. Ladefoged, J., and Winkler, K. Hemodynamics in acute renal failure. The effect of hypotension induced by dihydralazine on renal blood flow, mean circulation time for plasma, and renal vascular volume in patients with acute oliguric renal failure. *Scand. J. Clin. Lab. Invest.* 26: 83 (1970).
7. Flanigan, W. J., and Oken, D. E. Renal micropuncture study of the development of anuria in the rat with mercury-induced acute renal failure. *J. Clin. Invest.* 44: 449 (1965).
8. Oken, D. E., Arce, M. L., and Wilson, D. R. Glycerol-induced hemoglobinuric acute renal failure in the rat. I. Micropuncture study of the development of oliguria. *J. Clin. Invest.* 45: 724 (1966).
9. Thiel, G., et al. Glycerol induced hemoglobinuric acute renal failure in the rat. II. The experimental model, predisposing factors and pathophysiologic features. *Nephron* 4: 276 (1967).
10. Oken, D. E., DiBona, G. F., and McDonald, F. D. Micropuncture studies of the recovery phase of myohemoglobinuric acute renal failure in the rat. *J. Clin. Invest.* 49: 730 (1970).
11. Barenberg, R. L., et al. Clearance and micropuncture study of renal function in mercuric chloride treated rats. *J. Lab. Clin. Med.* 42: 473 (1968).
12. Ruiz-Guinazu, A., Coelho, J. B., and Paz, R. A. Methemoglobin-induced acute renal failure in the rat. *Nephron* 4: 257 (1967).
13. Jaenike, J. R.: Micropuncture study of methemoglobin-induced acute renal failure in the rat. *J. Lab. Clin. Med.* 73: 459-468, 1969.
14. Cirksena, W. J. Pathogenetic studies in a model of pigment nephropathy in the rat, In: *Pathogenesis and Clinical Findings with Renal Failure*. U. Gessler, K. Schroder, and H. Weidinger, Eds., Georg Thieme Verlag, Stuttgart, 1971.
15. Henry, L. N., Lane, C. E., and Kashgarian, M. Micropuncture studies of the pathophysiology of acute renal failure in the rat. *Lab. Invest.* 19: 309 (1968).
16. Steinhausen, M., Eisenbach, G. M., and Helmstadter, V. Concentration of lissamine green in proximal tubules of antidiuretic and mercury poisoned rats and the permeability of these tubules. *Pflügers Arch. Ges. Physiol.* 311: 1 (1969).
17. Bank, N., Mutz, B. F., and Aynedjian, H. S.: The role of "leakage" of tubular fluid in anuria due to mercury poisoning. *J. Clin. Invest.* 46: 695 (1967).
18. Flamenbaum, W., et al. Micropuncture study of renal

- tubular factors in low dose mercury poisoning. *Nephron* 8: 221 (1971).
19. DiBona, G. F., et al. Maintenance of renal function in salt loaded rats despite severe tubular necrosis induced by HgCl_2 . *Nephron* 8: 205 (1971).
 20. Owen, K., Desautels, R., and Walter, C. W. Experimental renal tubular necrosis—the effect of Pitressin. *Surg. Forum* 4: 459 (1953).
 21. Wilson, D. R., et al. The role of concentrating mechanisms in the development of acute renal failure. Micropuncture studies using diabetes insipidus rats. *Nephron* 6: 128 (1969).
 22. Goormaghtigh, N. Vascular and circulatory changes in renal cortex in the anuric crush-syndrome. *Proc. Soc. Exptl. Biol. Med.* 59: 303 (1945).
 23. Kokot, F., and Kuska, J. Plasma renin activity in acute renal insufficiency. *Nephron* 6: 115 (1969).
 24. DiBona, G. F., and Sawin, L. L. Renin-angiotensin system in acute renal failure in the rat. *Lab. Invest.* 25: 528 (1971).
 25. Gross, F., et al. The role of the renin-angiotensin system in blood pressure regulation and kidney function. *Can. Med. Assoc. J.* 90: 258 (1964).
 26. McDonald, F. D., et al. The prevention of acute renal failure in the rat by long-term saline loading. A possible role of the renin-angiotensin axis. *Proc. Soc. Exptl. Biol. Med.* 131: 610 (1969).
 27. Thiel, G., McDonald, F. D., and Oken, D. E. Micropuncture studies of the basis for prevention of renin depleted rats from glycerol induced acute renal failure. *Nephron* 7: 67 (1970).
 28. Flamenbaum, W., et al. Uranyl nitrate-induced acute renal failure in the rat: Micropuncture and renal hemodynamic studies. *Kidney Int.* 6: 408 (1974).
 29. Flamenbaum, W., Kotchen, T. A., and Oken, D. E. Effect of renin immunization on mercuric chloride and glycerol-induced renal failure. *Kidney Int.* 1: 406 (1972).
 30. Hicks, J. Sodium metabolism and blood flow distribution in the normotensive and hypertensive rat kidney. Doctoral thesis, Georgetown Univ., 1971.
 31. Kilcoyne, M. M., and Cannon, P. J. Neural and humoral influences on intrarenal blood flow distribution during thoracic caval occlusion. *Am. J. Physiol.* 220: 1231 (1966).
 32. Oken, D. E., et al. Active and passive immunization to angiotensin in experimental acute renal failure. *Kidney Int.* 7: 12 (1975).
 33. Powell-Jackson, J. D., et al. The effect of angiotensin II antisera and synthetic inhibitors of the renin-angiotensin system on glycerol-induced acute renal failure in the rat. In: *Proceedings of a Conference on Acute Renal Failure*, E. Friedman, Ed. DHEW Publication 74-608 1973.
 34. Hayes, J. M., et al. Resistance to glycerol induced hemoglobinuric acute renal failure. *Nephron* 7: 155 (1970).
 35. Oken, D. E., et al. Resistance to acute renal failure afforded by prior renal failure: Examination of the role of renal renin content. *Nephron* 15: 131 (1975).
 36. Wolf, C. J., and Oken, D. E. Does resistance to acute renal failure (ARF) reflect refractoriness to angiotensin? (abstract) *Clin. Res.* 22: 551A (1974).
 37. Daugharty, T. M., et al. Dynamics of glomerular ultrafiltration in the rat. V. Response to ischemic injury. *J. Clin. Invest.* 53: 105 (1974).
 38. Blantz, R. C. The mechanism of acute renal failure after uranyl nitrate. *J. Clin. Invest.* 55: 621 (1975).
 39. Zins, G. R. Renal prostaglandins. *Am. J. Med.* 58: 14 (1975).
 40. Barger, A. C., and Herd, J. A. Study of renal circulation in unanesthetized dog with inert gases. In: *Third International Congress of Nephrology*, Washington, D.C., 1966, Vol. I, S. Karger, Basel, Switzerland, 1966, p. 174.
 41. Jaffe, B. M., et al. Renal concentrations of prostaglandin E in acute and chronic renal ischemia. *Biochem. Biophys. Res. Commun.* 49: 799 (1972).
 42. Lonigro, A. J., et al. Differential inhibition by prostaglandins of the renal actions of pressor stimuli. *Prostaglandins* 3: 595 (1973).
 43. Fine, L. G. Acquired prostaglandin E_2 (medullin) deficiency as the cause of oliguria in acute tubular necrosis: a hypothesis. *Israel J. Med. Sci.* 6: 346 (1970).
 44. Gerhard, H., and Mulrow, P. J. The effect of acute renal failure on renal prostaglandin A (PGA) (abstract), *Clin. Res.* 22: 528A (1974).
 45. Torres, V. E., et al. Renal prostaglandin E during acute renal failure. *Prostaglandins* 8: 353 (1974).
 46. Torres, V. E., et al. Indomethacin enhancement of glycerol-induced acute renal failure in rabbits. *Kidney Int.* 7: 170 (1975).
 47. Anggard, E., et al. Subcellular localization of the prostaglandin system in the rabbit renal papilla. *Acta Physiol. Scand.* 84: 231 (1972).
 48. Larsson C. and Anggard, E. Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. *Eur. J. Pharmacol.* 21: 30 (1973).
 49. Oken, D. E. Hypothesis: on the role of prostaglandins in the pathogenesis of acute renal failure. *Lancet* 1:1319 (1975).
 50. Kauker, M. L. Tracer microinjection studies of prostaglandin E_2 transplant in the rat nephron. *J. Pharm. Exptl. Therap.* 193: 274 (1975).
 51. Frolich, J. C., et al. Urinary prostaglandins identification and origin. *J. Clin. Invest.* 55: 763 (1975).